

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

1. (Currently amended) An isolated post-transcriptional regulatory element (PRE) nucleic acid comprising SEQ ID NO:1, the PRE nucleic acid defined as having the following property:

(i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1, and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.

2. (Currently amended) An isolated nucleic acid comprising a post-transcriptional regulatory element (PRE) nucleic acid inserted into a nucleo-cytoplasmic transport element (NCTE)-deficient hybrid virus clone, the PRE nucleic acid defined as having the following properties:

(i) when an encoded PRE-containing hybrid human immunodeficiency virus (HIV)-1 ~~virus~~ infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs; and,

(ii) the PRE nucleic acid has at least 80% nucleic acid sequence identity to the sequence set forth in SEQ ID NO:1.

3. (Previously presented) The isolated nucleic acid of claim 2, wherein the PRE nucleic acid is inserted in place of a wild type nucleocytoplasmic transport element (NCTE).

4. (Previously presented) The isolated nucleic acid of claim 2, wherein the virus is a retrovirus.

5. (Previously presented) The isolated nucleic acid of claim 4, wherein the retrovirus clone is a HIV clone.

6. (Previously presented) The isolated nucleic acid of claim 5, wherein the PRE nucleic acid comprises the sequence set forth in SEQ ID NO:1.

7. (Previously presented) The isolated nucleic acid of claim 6, wherein when the PRE-containing hybrid HIV-1 virus infects activated huPBMCs, the level of expression of HIV-1 p24<sup>gag</sup> is between about 10 fold and about 50 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus infects activated huPBMCs.

8. (Currently amended) An expression cassette comprising a post-transcriptional regulatory element (PRE) nucleic acid operably linked to a promoter, wherein the PRE nucleic acid defined as having the following properties:

- (i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleocytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1, and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.; and,
- (ii) the PRE has at least 80% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

9. (Previously presented) The expression cassette of claim 8, wherein the PRE nucleic acid is SEQ ID NO:1.

10. (Previously presented) The expression cassette of claim 8, wherein the expression cassette is an expression vector.

11. (Previously presented) A transfected cell comprising an expression cassette of claim 8.

12. (Currently amended) A recombinant virus, wherein the virus either lacks or has non-functional endogenous post-transcriptional RNA nucleo-cytoplasmic transport elements (NCTEs), further comprising a post-transcriptional regulatory element (PRE) nucleic acid operatively inserted into the virus, the PRE nucleic acid capable of acting as an exogenous functional NCTE to reconstitute the lacking or non-functional endogenous NCTE and to reconstitute the infectivity of the virus in a mammalian cell,

wherein the PRE nucleic acid has at least 80% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

13. (Previously presented) The recombinant virus of claim 12, wherein the virus is a retrovirus.

14. (Previously presented) The recombinant virus of claim 12, wherein the PRE has at least 90% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

15. (Previously presented) The recombinant virus of claim 14, wherein the PRE comprises a sequence as set forth in SEQ ID NO:1.

16. (Previously presented) The recombinant virus of claim 12, wherein when the PRE-containing hybrid HIV-1 virus infects activated huPBMCs, the level of expression of HIV-1 p24<sup>gag</sup> is between about 10 fold and about 50 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus infects activated huPBMCs.

17. (Previously presented) The recombinant virus of claim 12, wherein the virus is HIV-1.

18. (Previously presented) The recombinant virus of claim 12, wherein the insertion of the PRE is in the 3' untranslated region of the virus.

19. (Previously presented) The recombinant virus of claim 17, wherein the insertion of the PRE is in or flanking the Nef region of the HIV-1 virus.

20. (Previously presented) The recombinant virus of claim 17, wherein the HIV-1 further lacks a functional Nef.

21. (Currently amended) An immunogenic composition ~~A vaccine for the prophylaxis or amelioration of a viral infection in a mammal~~ comprising an attenuated retrovirus, wherein the attenuated retrovirus, when administered ~~as a vaccine~~ in sufficient amounts is capable of eliciting an immune response to the retrovirus in a mammal with a functional immune system,

wherein the attenuated retrovirus lacks an endogenous functional post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE) and/or the ability to express an endogenous functional NCTE binding protein, and the attenuated retrovirus further comprises a post-transcriptional regulatory element (PRE) nucleic acid defined as having the following properties:

- (i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1, and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.; and,
- (ii) the PRE has at least 80% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

22. (Currently amended) The immunogenic composition ~~vaccine~~ of claim 21, wherein the attenuated retrovirus is HIV-1.

23. (Currently amended) The immunogenic composition ~~vaccine~~ of claim 21, wherein the insertion of the PRE is in the 3' untranslated region of the virus.

24. (Currently amended) The immunogenic composition ~~vaccine~~ of claim 22, wherein the insertion of the PRE is in or flanking the Nef region of the HIV-1 virus.

25. (Currently amended) The immunogenic composition ~~vaccine~~ of claim 22, wherein the attenuated HIV-1 further lacks a functional Nef.

26. (Currently amended) A kit for ~~the prophylaxis or amelioration of~~ eliciting an immune response to a virus infection in a mammal, the kit comprising an immunogenic composition ~~a vaccine~~ and a pharmacologically acceptable carrier, wherein the immunogenic composition ~~vaccine~~ comprises an attenuated retrovirus,

wherein the attenuated retrovirus, when administered ~~as a vaccine~~ in sufficient amounts is capable of eliciting an immune response to the retrovirus in a mammal with a functional immune system,

wherein the attenuated retrovirus lacks an endogenous functional post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE) and/or the ability to express an endogenous functional NCTE binding protein, and the attenuated retrovirus further comprises a post-transcriptional regulatory element (PRE) nucleic acid defined as having the following properties:

(i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1, and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.; and,

(ii) the PRE has at least 80% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

27. (Currently amended) The kit of claim 27, further comprising an instructional material teaching the use of the immunogenic composition vaccine, wherein the instructional material indicates that the immunogenic composition vaccine is used for ~~the prophylaxis or amelioration of~~ eliciting an immune response to HIV-1 infection in a mammal; that the immunogenic composition vaccine is to be administered to a mammal in a therapeutically effective amount sufficient to express a viral protein; wherein the immunogenic composition vaccine will not cause clinically significant CD4+ cell depletion; and, the expression of the viral protein elicits an immune response to the attenuated HIV-1 virus.

28-29. (Canceled)

30. (Currently amended) A method for eliciting an immune response to a virus in a mammal, comprising administering to a mammal a therapeutically effective amount of an attenuated recombinant virus, wherein the recombinant virus comprises a post-transcriptional regulatory element (PRE) defined as having the following properties:

(i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleocytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1, and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.; and,

(ii) the PRE has at least 80% nucleic acid sequence identity to the sequence as set forth in SEQ ID NO:1.

31. (Withdrawn) A method of identifying functional PREs, the method comprising,

- (i) providing a PRE-deficient virus unable to replicate in a cell line;
- (ii) ligating nucleic acid fragments into a genome of the virus, thereby constructing a recombinant viral clone;
- (iii) inserting the recombinant viral clone into the cell line; and
- (iv) isolating a nucleic acid comprising a functional PRE from the recombinant viral clone that is propagated in the cell line.

32. (New) The method of claim 30, wherein the virus is HIV-1.

33. (New) An isolated post-transcriptional regulatory element (PRE) nucleic acid comprising SEQ ID NO:1, the PRE nucleic acid defined as having the following property:

- (i) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE), is capable of functioning as a NCTE in the hybrid HIV-1 to increase its protein expression and to produce functional virus; and

- (ii) the PRE nucleic acid, when inserted in a recombinant, hybrid human immunodeficiency virus (HIV)-1 lacking or having a non-functional wild-type post-transcriptional RNA nucleo-cytoplasmic transport element (NCTE), and when the PRE-containing hybrid HIV-1 virus infects activated human peripheral blood mononuclear cells (huPBMCs), the level of expression of HIV-1 p24<sup>gag</sup> is between about 5 fold and about 200 fold less than levels of p24<sup>gag</sup> expression when HIV-1 wild type virus, utilizing wild-type NCTE, infects activated huPBMCs.